

# First approach to the study of atresia in the ovary of sardine, *Sardina pilchardus* (Walb.)

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## ABSTRACT

The ovary atresia characteristics of sardine, *Sardina pilchardus*, on the Iberian Peninsula were described using the same nomenclature and criteria adopted for the northern anchovy, *Engraulis mordax* J., by Hunter and Macewicz (1985a). The data used were based on sardine biological information obtained from laboratory experiments and Daily Egg Production Method (DEPM) survey samples.

In the laboratory, the occurrence of atresia was low, and most atresia stages corresponded to  $\alpha$ -stage. An increase in the number of females in more advanced degeneration stages was noted as starvation time increased.

In the natural population, atresia stages mostly appeared after the complete degeneration of postovulatory follicles. The complete yolked oocyte atresia process took no more than 6 days.

**Key words:** Atresia, sardine, Iberian Peninsula.

## RESUMEN

Estudio preliminar de atresia en el ovario de sardina, *Sardina pilchardus* (Walb.).

Se describen las características del proceso de atresia en el ovario de *Sardina pilchardus*, de la península Ibérica, usando los mismos criterios adoptados para la anchoa, *Engraulis mordax* J., por Hunter and Macewicz (1985a). Los datos utilizados proceden de experimentos realizados en cultivo de laboratorio y de las muestras de campaña del Método de Producción Diaria de Huevos (DEPM) de 1988. La presencia de atresia en los ovarios de sardinas mantenidas en laboratorio es baja y la mayoría de los estados de atresia son del tipo de  $\alpha$ -atresia. Se produjo un incremento del número de hembras con estados más avanzados de atresia al ser mantenidas éstas en inanición.

Los datos de sardinas de la población demuestran que el porcentaje de ovarios con ovocitos vitelados atrésicos se incrementa después de la completa degeneración de los folículos postovulatorios. El proceso completo de degeneración de estos folículos no supera el período de 6 días.

**Palabras clave:** Atresia, sardina, península Ibérica.

## INTRODUCTION

The importance of atresia studies for a better understanding of reproductive biology of species, in particular for the determination of the reproductive state of female fish, has been widely recognized.

The interpretative power of histological analysis could be enhanced if the processes of ovarian atresia were better documented.

In serial spawning fishes, oocyte atresia (degeneration of oocytes) occurs throughout the spawning season, but becomes marked as the spawning season closes, and the remaining advanced oocytes in the ovary are resorbed.

A general knowledge of atresia processes is required to determine the age of postovulatory follicles, since some follicular atresia stages are very similar in appearance to late-stage postovulatory follicles. It is also an histological marker for spawning cessation, and as a consequence it can be used to determine the optimal cruise period for Daily Egg Production Method (DEPM) biomass estimation. Atresia information is also necessary to establish the first maturity size, and can be used to separate immature females from those in postspawning condition, which the DEPM requires.

## MATERIAL AND METHODS

The material used in this paper is based on sardine biological information obtained in a laboratory experiment and DEPM survey samples.

### Laboratory Experiment

Adult sardine were captured by commercial purse seine during February 1989. Only 15 females, averaging 17.1 mm  $L_t$ , were kept in the laboratory and held in a 9 m<sup>3</sup> pool (1 m deep). Samples of five females were taken at 13, 15 and 17 days after captivity. The temperature of the seawater ranged from 13.3 to 14.7 °C. During this time, fish were submitted to starvation in order to trigger ovary resorption.

We assumed that all the females had active ovaries at the time of capture without

atresia, since no degeneration processes were observed in females sampled from 19-61 hours after capture, although only three females were available.

During the laboratory experiment, all sampled females were measured, their ovaries extracted and a small ovary piece was taken for histological analysis. The ovaries were fixed in 10 % neutral buffered formalin and embedded in paraffin. Histological sections were cut at 6  $\mu$ m and stained with hematoxylin-eosin (Hunter, 1985).

### Sea Data

The ovaries of sardine taken in DEPM trawl surveys used for biomass estimation in 1978 and 1990 (García *et al.*, 1991, 1992) were histologically examined. In all collections, ovaries were classified according to atresia characteristics, as well as on the basis of the presence of postovulatory follicles (Day-0 and Day-1) according to the methods described by Hunter and Macewicz (1985a) and Hunter and Goldberg (1980). In order to compare the atresia data, all the female samples were obtained off the Spanish Atlantic coast (633 in 1988 and 369 in 1990), since during 1990 no DEPM survey was carried out off the Portuguese coast.

## RESULTS, DISCUSSION AND CONCLUSIONS

The histological characteristics of different atresia classes are described below. The nomenclature and general characteristics used to differentiate the atresia stages follow those established for the northern anchovy, *Engraulis mordax*, by Hunter and Macewicz (1985a).

### Alpha-atresia ( $\alpha$ -atresia)

In sardine, the initial stages of atresia present similar histological characteristics to  $\alpha$ -atresia, as described by Hunter and Macewicz (1985a).

In yolked oocytes, the first step of atresia is characterized by the disintegration of the nucleus, evident by an irregular shape and



dark basophilic staining. The zona radiata slowly dissolves, which is indicated by the loss of striation and uneven diameter (fig. 1).

Later on, granulosa cells enlarge. The zona radiata breaks and granulosa cells invade the degenerating oocyte. At this time,

slides from sea-caught females, and was mainly characterized by the disintegration of zona radiata and of yolk plates (fig. 3). The weak occurrence of atresia in hydrated oocytes may indicate that, in sardine, nearly all hydrated oocytes are ovulated.

The end of this stage, in both yolked and

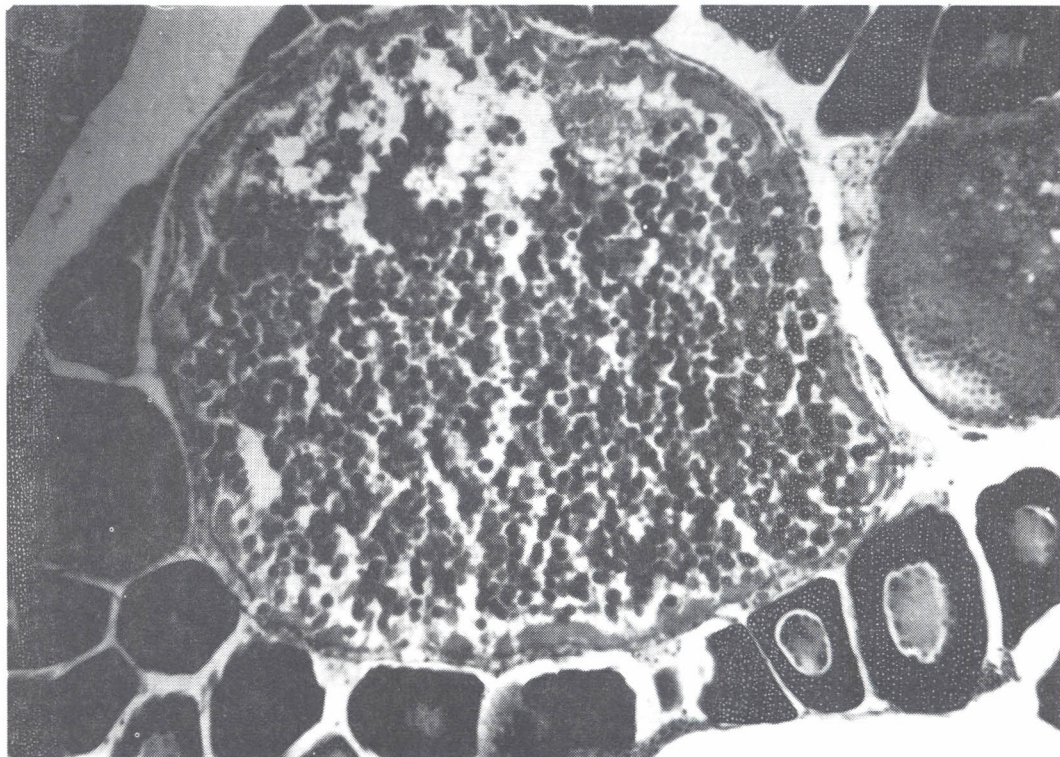


Fig. 1.—Initial Alpha-atresia ( $\alpha$ -atresia) in yolked oocytes.

Fig. 1.—Inicio de Alpha-atresia ( $\alpha$ -atresia) en ovocitos vitelados.

some disintegration of yolk globules can be observed, especially those yolk globules located outside the zona radiata. Yolk desintegration is indicated by less refractive globules, fused globules or expanded globules with a less regular shape (fig. 2).

Also during this stage, thecal tissue remains as a thin layer covering the granulosa cells. This layer becomes well irrigated by the presence of numerous blood cell capillaries.

In unyolked oocytes,  $\alpha$ -atresia is similar, but without yolk. The nucleus disintegrates, the zona radiata, if present, dissolves, and the granulosa cells enlarge.

Atresia in hydrated oocytes was only observed in three of all ovary histological

unyolked oocytes, occurs when the resorption is completed, and all that remains is the atretic follicle. The entire oocyte is reabsorbed by the hypertrophing granulosa cells of the follicle (Hunter and Macewicz, 1985a).

### Beta-atresia ( $\beta$ -atresia)

In the second stage of atresia, major degeneration and resorption of the follicle (granulosa and thecal cells) occurs. At the beginning of this stage, the atretic follicle is a compact structure composed of numerous disorganized granulosa cells surrounded by a thin thecal and blood vessel layer (fig. 4).



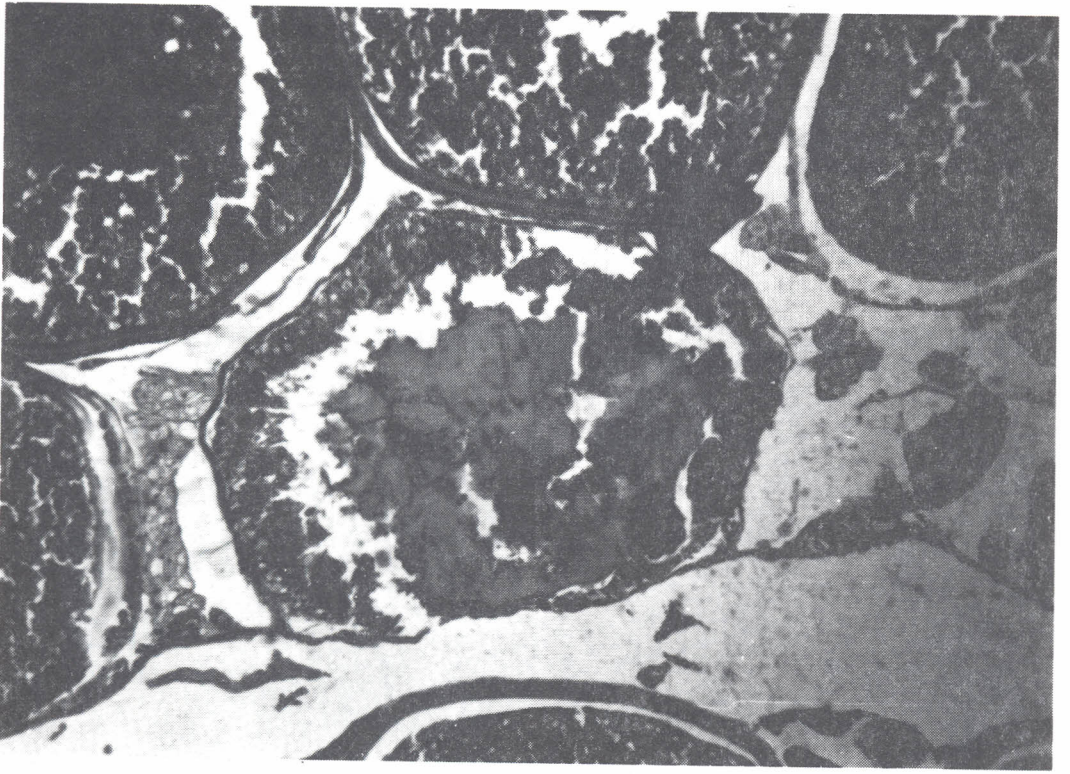


Fig. 2.—Advance  $\alpha$ -atresia.

Fig. 2.—Estado avanzado de  $\alpha$ -atresia.

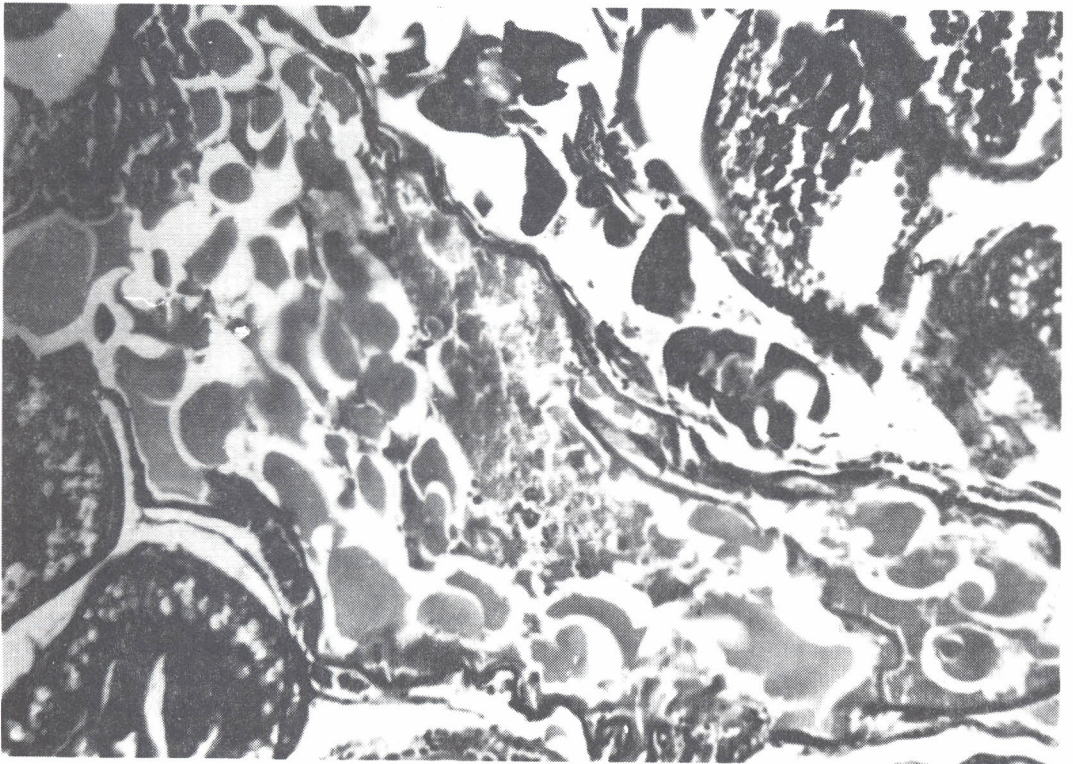


Fig. 3.—Atresia in hydrated oocyte.

Fig. 3.—Atresia en un ovocito hidratado.



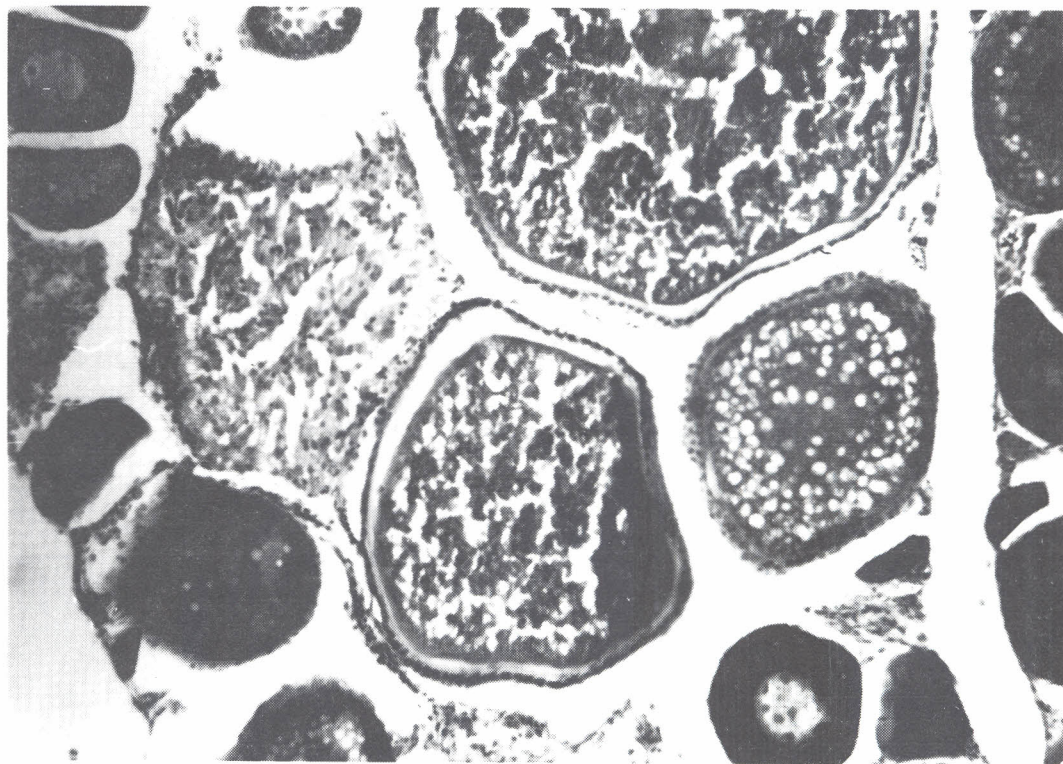


Fig. 4.—Beta-atresia ( $\beta$ -atresia) in sardine.

Fig. 4.—Beta-atresia ( $\beta$ -atresia) en sardina.

The nuclei of some granulosa cells are pycnotic, and spherical intercellular cavities exist among the granulosa cells, which are remnants of the oil droplet, which takes longer than yolk to resorb, and in H & E sections appear empty (Hunter, Macewicz and Sibert, 1986).

In the Iberian sardine, the shapes of intercellular cavities and of lumen postovulatory follicles more than 48 hours old are different, which make them easily distinguishable. On the other hand, in the northern anchovy (species with no oil droplet in their eggs (Moser and Ahlstrom, 1985)), the shape of both structures are similar.

According to Hunter and Macewicz (1985a) three patterns of atresia may occur at the end of  $\beta$ -stage:

- follicle may follow the classic pattern and pass through subsequent  $\gamma$  and  $\delta$ -atresia (Bretshneider and Duyevene de Wit, 1947 in Hunter and Macewicz, 1985a);

- follicle may be completely resorbed during  $\beta$ -stage, leaving no histological characteristics that can be identified;
- the follicle may pass directly from a  $\beta$ -stage to an  $\delta$  structure without passing to the intervening  $\gamma$ -stage.

The ovary analysis of sea-caught female sardine led us to the realization that follicles were completely resorbed during  $\beta$ -stage. However, in those observations we recognized some histological structures that we did not originally associate with atresia. After analyzing the ovary atresia stages from starved sardine kept in the laboratory, a correspondence of those unknown histological structures with more  $\beta$ -stage advanced grades of atresia was accepted.

#### **Gamma-atresia ( $\gamma$ -atresia)**

At this stage, the atretic follicle presents the same relative size as in  $\beta$ -stage, but is

less stained. The granulosa and thecal cells are still visible. Blood cells continue to surround the thecal layer (which at this stage is very thin). The intercellular cavities are still present and are larger than in  $\beta$ -stage (fig. 5).

The general aspect of this stage seems to be much closer to the one with an unusual appearance described by Hunter and Macewicz (1985b): "the flocculent yellow material is extracellular rather than intracellular, and the material is encapsulated by a layer of granulosa and thecal cells".

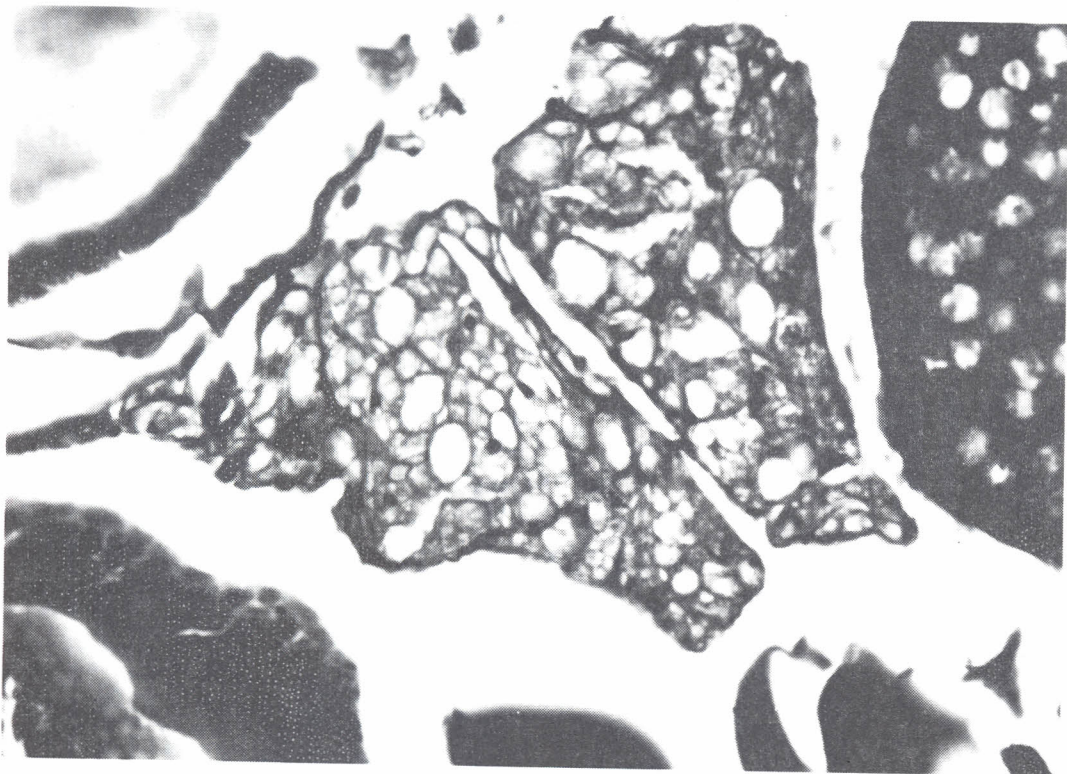


Fig. 5.—Gamma-atresia ( $\gamma$ -atresia) in sardine.

Fig. 5.—Gamma-atresia ( $\gamma$ -atresia) en sardina.

#### Delta-atresia ( $\delta$ -atresia)

During the last stage of atresia, the size of the atretic follicle is very reduced, and light eosinophilic staining can be seen.  $\delta$ -stage appears as a network of filamentous material in which dark basophilic structures are suspended.

The number of granulosa cells and nuclei are also greatly reduced. Thecal and blood

vessels do not encompass the granulosa cells, probably because they have been absorbed. Intercellular cavities have disappeared (fig. 6).

#### Rates of atresia

The evaluation of rates at which sardine yolked and unyolked oocytes are reabsorbed, based on laboratorial work, created many difficulties. The insufficient number

of surviving females made it difficult to establish atresia rates for this species.

Three females were used to verify the occurrence of atresia at the time of capture. In females sampled from 19 to 61 hours after capture, no degeneration processes were observed, which led us to assume that all the sea-caught females had active ovaries without any atresia.

Ovary samples of starved females were



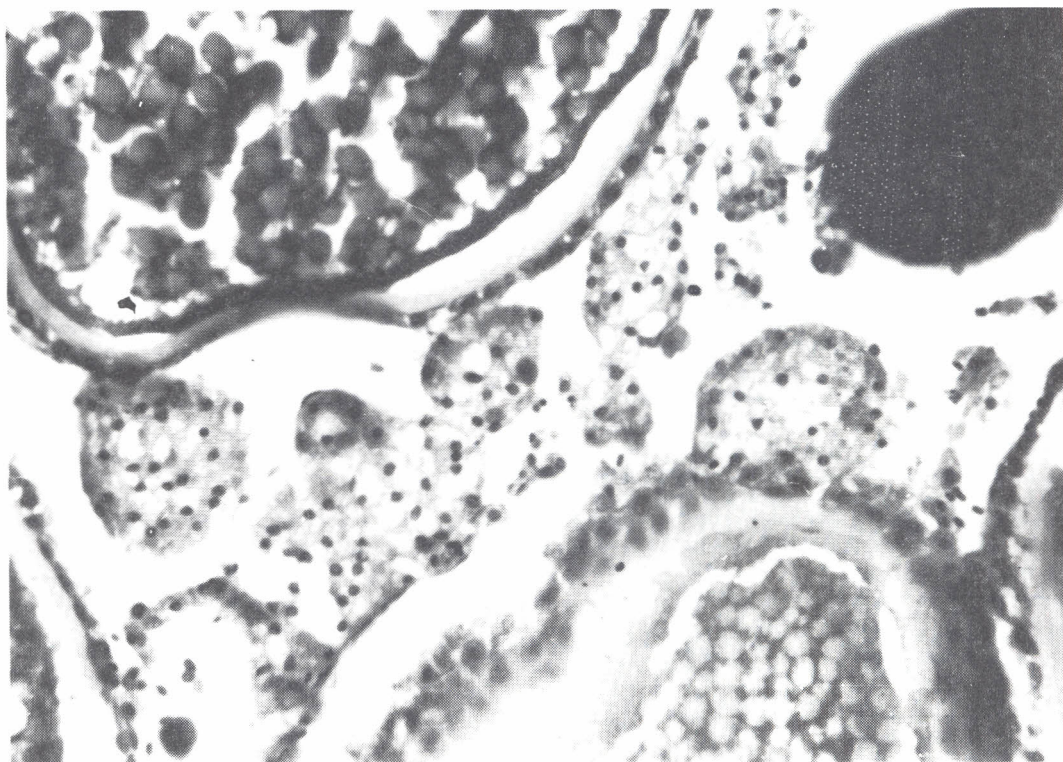


Fig. 6.—Delta-atresia ( $\delta$ -atresia) in sardine.

Fig. 6.—Delta-atresia ( $\delta$ -atresia) en sardina.

taken at three different elapsed times from capture: 13, 15 and 17 days. Examining those samples, we noted that atresia in ovaries was not very marked and also that most atretic stages corresponded to  $\alpha$ -stage (table 1). A tendency towards an increase in the number of females in more advanced degeneration stages as starvation time lengthened was also noted.

In the first three sampled females (elapsed time from onset of starvation = 2 days), no atresia was observed. By the 13th day, 40% of the 5 females were in  $\alpha$ -stage oocyte resorption. At the 15th day, all the females with atretic ovaries presented more than 50% of yolked oocytes in  $\alpha$ -stage and one of them also presented oocytes in  $\beta$ -atresia stage. This may indicate that after this time the process of ovary reabsorption is at a level that spawning could no longer take place.  $\gamma$  and  $\delta$ -stages were less common, appearing only after 17 days of starvation.

The resorption of unyolked oocytes began just at the 15th day of starvation (table 1). Throughout the rest of that starvation period, some of the females had some unyolked oocytes in  $\alpha$ -atresia indicating a continual recruitment of atretic follicles from the unyolked oocyte classes.

#### Natural Atresia Presence

In this section, we analyze sea data taken in 1988 and 1990 for the occurrence of four ovarian atretic states (Hunter and Macewicz, 1985 b) in Iberian sardine.

Atresia state 0 No  $\alpha$ -atresia of yolked oocytes (yolked oocytes present).

Atresia state 1  $\alpha$ -atresia in yolked oocytes where <50% of yolked oocytes are affected.

Table 1.—Absolute frequency (in numbers of females) in different atresia stages during starvation.

Tabla 1.—Frecuencia absoluta (en número de hembras) en diferentes estados de atresia durante el período de inanición.

Elapsed time (days)	N	No atresia	$\alpha$			$\beta$	$\gamma + \delta$
			unyolked	yolked < 50 %	oocyte > 50 %		
2	3	3	0	0	0	0	0
13	5	3	0	1	1	1	0
15	5	3	0	1	1	1	0
17	5	1	1	0	2	1	1

Table 2.—Absolute frequency (in numbers) of mature female sardine (with yolked oocytes in their ovaries) by atretic and reproductive state.

Tabla 2.—Frecuencia absoluta (en número) de hembras maduras (con presencia de ovocitos vitelados en el ovario) según los diferentes estados de atresia y desarrollo reproductivo.

Collection date	Atresia stage	Hydrated oocytes	Postovulatory follicles		No spawning evidence
			Day-0	Day-1	
1988	0	177	74	46	202
	1	1	1	1	41
	2	0	2	0	19
1990	0	71	74	39	209
	1	0	0	2	11
	2	0	0	0	2

Atresia state 2     $\alpha$ -atresia in yolked oocytes where 50 % or more of yolked oocytes.

In addition to the atretic condition of the ovary, we also included histological evidence of recent or imminent spawning, using the system of Hunter and Goldberg (1980), i.e., presence of hydrated eggs (imminent spawning), Day-0 or new postovulatory follicles (spawning on the night of capture), and Day-1 postovulatory follicles (spawning on the night before capture). We also include the number of mature females with no recent spawning evidence in their ovaries, i.e., with yolked oocytes but with no postovulatory follicles. All data on the incidence of reproductive states are given in table 2.

Of the females classed in atresia state 1 (females with < 50 % of the yolked oocytes in  $\alpha$ -stage of atresia), 3 % showed evidence of recent or imminent spawning (postovulatory follicles or hydrated oocytes); 44 % of the females without atresia showed evidence of spawning. This indicates that a relatively large number of females in atresia state 1 spawn, despite the atretic condition of their ovaries. On the contrary, only 10 % of those in atresia state 2 (females with 50 % or more of the yolked oocytes in  $\alpha$ -stage of atresia) had recently been reproductively active.

The 91 % of mature females with no evidence of spawning were classified in atresia states 1 and 2. The comparison of these figures with those from active females show that atretic states appear mostly after com-



plete degeneration of postovulatory follicles. Assuming that during the active spawning period sardine spawn every 8th day (García *et al.*, 1991), the complete yolked oocyte atretic process will last no longer than 6 days.

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